Astrocyte dysfunction in neurological disorders: a molecular perspective

Gerald Seifert, Karl Schilling‡ and Christian Steinhäuser**

Abstract | Recent work on glial cell physiology has revealed that glial cells, and astrocytes in particular, are much more actively involved in brain information processing than previously thought. This finding has stimulated the view that the active brain should no longer be regarded solely as a network of neuronal contacts, but instead as a circuit of integrated, interactive neurons and glial cells. Consequently, glial cells could also have as yet unexpected roles in the diseased brain. An improved understanding of astrocyte biology and heterogeneity and the involvement of these cells in pathogenesis offers the potential for developing novel strategies to treat neurological disorders.

Complex glial cells
Cells in the postnatal brain that express various types of voltage- and time-dependent ion channels as well as transmitter receptors. They do not generate action potentials and have been shown to express S100β.

**Department of Experimental Neurobiology, Clinic of Neurosurgery, University of Bonn, Sigmund-Freud-Straße 25, D-53105 Bonn, Germany. ‡Department of Anatomy and Cell Biology, University of Bonn, Nussallee 10, D-53115 Bonn, Germany. Correspondence to C.S. e-mail: Christian.Steinhaeuser@ukb.uni-bonn.de*
doi:10.1038/nrn1870

Until about 20 years ago, astrocytes were regarded as electrically silent elements, lacking transmitter receptors and expressing a limited set of ion channels. Consequently, it was assumed that they do not participate in information processing. However, this view was challenged by a series of studies in cell culture that showed astrocytes have the potential to express a large variety of functional transmembrane proteins. In the early 1990s, studies on acute brain slice preparations revealed that subpopulations of astrocytes and neurons do indeed share almost the same set of channels and receptors. This led to the conclusion that astrocytes have the machinery to sense and respond to neuronal activity, but also raised the fundamental question of whether glial receptors are stimulated under physiological conditions and what types of event are triggered by such activation. In 1994, Haydon and colleagues[1] made the seminal observation that increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured astrocytes induces the release of glutamate, which demonstrated that astrocytes respond to activation and have active modulatory roles in intercellular communication. Subsequent studies *in situ* corroborated these findings and led to the view that astrocytes are direct communication partners of neurons and dynamically interact with synapses through the uptake and release of neurotransmitters and receptor-mediated intracellular Ca^{2+} signalling.

Despite the growing realization of the importance of glial cells in brain signalling, we are still in the initial stages of understanding the physiological consequences of the intriguing bidirectional communication between neurons and glial cells. Accordingly, although the number of conditions known to involve defective astrocytes is rapidly increasing, for most conditions it remains unclear whether the glial changes are causative of the disease or merely represent an accompanying phenomenon. This makes it difficult to distinguish pathologically 'relevant' from 'less relevant' alterations. Furthermore, it is becoming increasingly clear that there are different types of cell with astroglial properties within a given brain region, and that the properties of these cells can vary in different subregions. It is important to note that we have only rudimentary understanding of this astroglial diversity. These cell types are likely to be affected differently in different neurological disorders with distinct pathological consequences. In the past, most studies describing astroglial alterations in brain diseases did not identify the specific cellular subtypes affected. Consequently, we refer to several types of cell with astroglial properties as 'astrocytes'. These include the complex[2] and GluR-type glial cells[3–5], which co-express glial and neuronal markers and do not match the classical definition of an astrocyte.

This review focuses on disease-related alterations in astrocyte function. These alterations include: changes in the expression of plasma membrane ion channels, receptors, transporters and gap junction coupling; changes in intracellular Ca^{2+} signalling; and changes in astroglial transmitter metabolism and release. Inflammation-related processes (for example, changes in the synthesis and release of cytokines, chemokines, nitric oxide (NO) synthase (NOS) and oxygen free radicals) are not dealt with systematically, except for those cases in which interference with these pathways is paramount. After introducing basic properties of astrocyte physiology and general features of disease-related alterations in these cells, we highlight the growing evidence that implicates astrocyte dysfunction in the pathogenesis of certain neurological disorders.

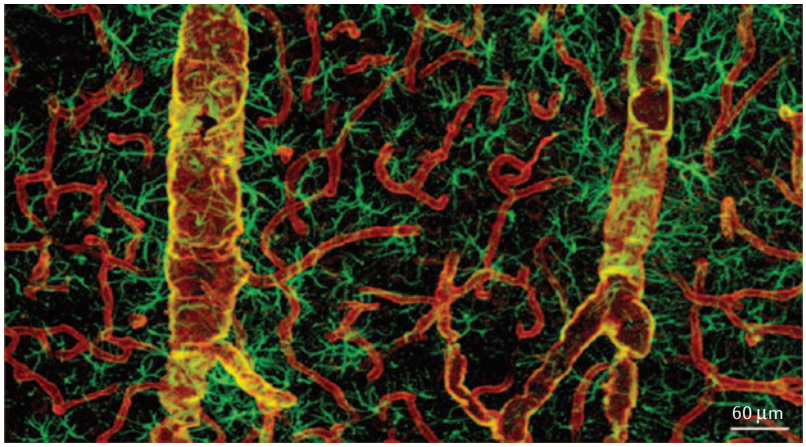


Figure 1 | **Cortical astrocytes labelled for glial fibrillary acidic protein and aquaporin 4.** Staining for glial fibrillary acidic protein (GFAP; green) reveals the typical, name-giving star-shaped arrangement of the intermediate-filament cytoskeleton in astrocytes. The subcellular segregation of aquaporin 4 (AQP4; red) to astroglial end-feet, which cover small blood vessels, documents a high degree of functional polarization. Reproduced, with permission, from REF. 165 © (2003) Society for Neuroscience.

Astrocyte physiology and neurological disorders

Astrocytes express almost the same set of ion channels and receptors as neurons[6,7], although the relative strength of expression varies between these cell types. For example, the density of K^+ channels in astrocytes far exceeds that of Na^+ channels, preventing the generation of glial action potentials. Nevertheless, a glia-specific, or at least preferential, expression has been shown for some of these channels and carriers. Among them is a subunit that belongs to the family of inwardly rectifying K^+ channels (Kir channels) — Kir4.1. In the CNS, this channel is predominantly localized at distant astrocytic processes that surround synapses or capillaries[8]. Recent work suggests a co-localization of Kir4.1 with the water channel aquaporin 4 (AQP4), which in the brain and spinal cord is also expressed by astrocytes (FIG. 1) but not by neurons. Increasing evidence indicates that a coordinated action of both channels is required for the astrocytes to maintain K^+ and water homeostasis in the CNS[9,10]. As discussed below, dysfunction of these astroglial transmembrane channels seems to have an important role in some neurological disorders (BOX 1).

In contrast to most mature neurons, astrocytes are usually coupled through gap junctions to form large intercellular networks. Astrocytic gap junctions are mainly formed by connexins 43 and 30 (CX43 and CX30, respectively) in a cell-type-specific fashion. Through these networks, astrocytes can dissipate molecules, such as K^+ or glutamate, and this is thought to be important to prevent the detrimental extracellular accumulation of these molecules[11]. Connexins also contribute to the propagation of intercellular Ca^{2+} waves, presumably by enhancing the release of ATP rather than providing an intercellular pathway for signal diffusion[12]. However, the pathological effect of disturbed expression of astroglial gap junctions is not well understood.

Another key function of astrocytes is the removal of neurotransmitters that are released by active neurons. Uptake of glutamate is accomplished by two glia-specific transporters — excitatory amino acid transporter 1 (EAAT1) and EAAT2 (which in rodents are known as glutamate–aspartate transporter (GLAST) and glutamate transporter 1 (GLT1), respectively). The activity of these transporters might dictate the kinetics of receptor currents at some synapses[13,14]. Compelling evidence suggests that disturbed glutamate uptake by astrocytes is directly involved in the pathogenesis of some neurological disorders (see below and BOX 2).

However, astrocytes can also release neuroactive agents, including the neurotransmitters glutamate, ATP and serine. The underlying mechanisms of astroglial transmitter release are not clear, but several studies indicate that the process is dependent on an increase in astroglial $[Ca^{2+}]_i$. Intriguingly, it seems that regulated, Ca^{2+}-dependent exocytosis — a mechanism that was thought to be exclusive to neurons in the CNS — contributes to transmitter release. Neuronal stimulation can increase astroglial $[Ca^{2+}]_i$ through activation of ionotropic and/or metabotropic glutamate receptors (iGluRs and mGluRs, respectively), $GABA_B$ (γ-aminobutyric acid type B) receptors or acetylcholine receptors, which implies the existence of manifold bidirectional communication between astroglia and neurons (reviewed in REF. 15). Interestingly, a recent report shows that a subtype of cell with astroglial properties receives direct synaptic input from glutamatergic and GABA-containing neurons[3].

What is the functional significance of transmitter release from astrocytes? Several reports suggest that these 'gliotransmitters' might modulate the strength of inhibitory and excitatory synaptic transmission by activating receptors in neurons[15]. Importantly, as astrocytes have fine terminal processes, each astrocyte can reach thousands of synapses simultaneously, and the release of gliotransmitters might lead to the synchronization of neuronal firing patterns. Rapid forms of neuron–glia interactions might also be involved in the regulation of local blood flow, as shown in cortical brain slices; neuronal stimulation led to glutamate release, activation of mGluRs in astrocytes and regulation of the tone of the vessels that are contacted by the stimulated astrocytic processes[16–18]. Emerging evidence indicates that disturbances of these mechanisms might be involved in the pathogenesis of neurological disorders (see below).

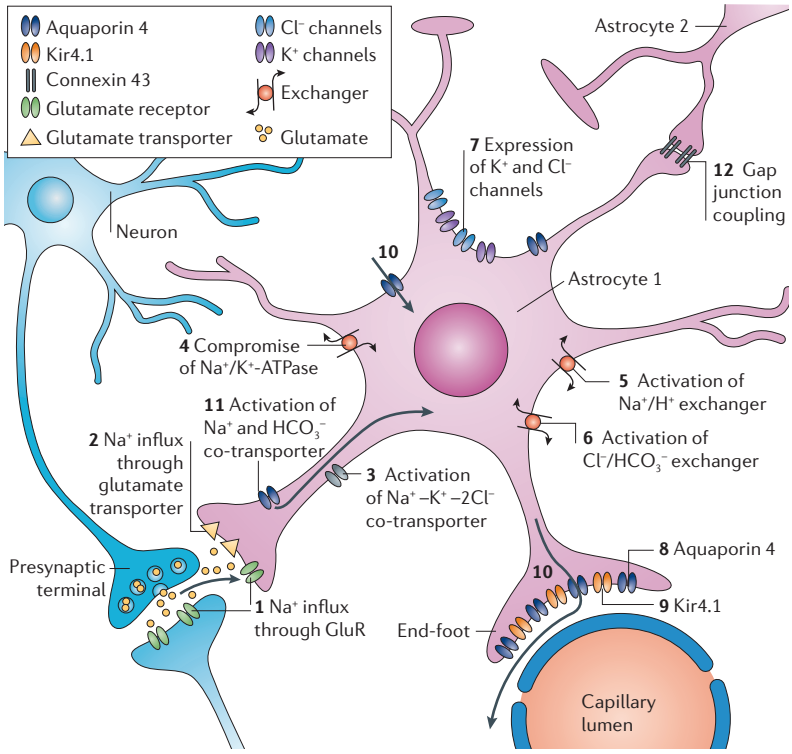
Several important aspects of neuron–glia interactions are not yet understood. For example, although recent findings corroborate the finding that astrocytes are heterogeneous with respect to antigen profiles and functional properties[4,5], it is not clear which types of astrocyte are activated and are capable of releasing transmitters, which transmitters can be released by astrocytes, which release mechanisms are used, or whether the efficiency of neuron–glia signalling changes during development. The specific role of different astrocyte subtypes in neurological disorders remains to be determined.

GluR-type glial cells
Cells in the postnatal hippocampus that express voltage- and time-dependent ion channels and glutamate receptors of the AMPA type. These cells receive synaptic input from glutamatergic and GABA-containing neurons, and lack gap junctional coupling as well as functional glutamate transporters. They express glial fibrillary acidic protein transcripts and S100β protein and probably represent a subpopulation of the complex glial cells.

Gap junctions
Gap junctions are channels composed of two juxtaposed hexameric hemichannels (connexons) forming intercellular pathways for the diffusion of ions and small molecules.

Inwardly rectifying K^+ channels
(Kir channels). These comprise a large family of transmembrane proteins that allow preferred passage of K^+ at negative membrane potentials.

Box 1 | **Swelling-related mechanisms**



Oedema (tissue swelling) results in marked changes of relative volumes occupied by cellular elements, as well as shrinkage and functional fragmentation of extracellular space[152]. The inappropriate redistribution of water and ions defining oedema results in cellular dysfunction and exacerbation of the initiating damage. In the retina, increased glutamate levels produce neuronal swelling and changes of Müller glia morphology and the extracellular space[153]. Here, neuronal swelling seems to result from Na^+ influx through AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and kainate receptors (**1**), and from Cl^- influx and water entry. Glial swelling after exposure to glutamate could result from Na^+ influx, which drives glutamate transporters[154] (**2**). High extracellular concentrations of K^+ ($[K^+]_o$) can cause swelling through Na^+–K^+–$2Cl^-$ co-transporters[155] (**3**). Astroglial swelling involves many of the mechanisms also observed in other cell types[156], including compromise of the Na^+/K^+-ATPase (**4**), activation of Na^+/H^+ exchanger (**5**), Cl^-/HCO_3^- exchangers (**6**) and Na^+–K^+–$2Cl^-$ co-transporters (**3**). As Na^+–K^+–$2Cl^-$ co-transporters allow the passage of ammonium ions, it is presumed that they contribute to oedema in hepatic encephalopathy. Various K^+ and Cl^- channels are expressed in swelling astrocytes[157] (**7**). Among neural cells, aquaporin 4 (AQP4) (**8**) is exclusively expressed by astrocytes[158], which suggests a molecular basis for astrocyte-specific mechanisms in oedema formation and that astrocytes could be potential therapeutic targets. The enrichment of AQP4 in astroglial end-feet surrounding blood vessels suggests that it regulates not only astrocyte volume, but also the exchange between vascular and interstitial compartments[10], as well as the size, shape and diffusion characteristics of the extracellular space[152]. The co-localization of AQP4 (**8**) and Kir4.1 (REF. 159) (**9**), the conspicuous changes in extracellular volume during spatial K^+ buffering[160] and the observation that K^+ clearance is delayed in animals that lack perivascular AQP4 (REF. 161) indicate that AQP4 is crucial to activity-dependent K^+ buffering[9,162]. Accordingly, activity-driven increases of $[K^+]_o$ cause depolarization of adjacent astrocytes, which favours astroglial uptake of Na^+ and bicarbonate (HCO_3^-) through Na^+ and HCO_3^- co-transporters (**11**). The resulting increase in intracellular osmolarity drives water into astroglia through AQP4 channels. This scenario is supported by the observation that stimulation-induced increase of $[K^+]_o$ is blunted in α-syntrophin-[161] and AQP4-null mice[26]. AQP4-mediated astroglial coupling of the extracellular and perivascular space is needed to ensure activity-related water flux. Disturbance of this flux brings about dilution of $[K^+]_o$[162]. A hint at the complexity and specificity of volume regulation under pathophysiological conditions can be gained from the observation that ablation of AQP4 can exacerbate or attenuate tissue damage[89,163,164]. Spatial buffering of ions and water handling is also dependent on astrocyte gap junctional coupling (**12**).

Epilepsy

Epilepsy is one of the most prevalent neurological diseases, affecting about 1% of the population worldwide. It is characterized by repetitively occurring seizures, that is, transient disturbances of neuronal function associated with motor behavioural abnormalities and disturbances of consciousness, which have devastating behavioural, social and occupational consequences. The seizures might also damage the brain and increase pre-existing neurological deficits. Currently available anticonvulsant therapies do not control seizures in about a third of epileptic patients.

Epilepsy is often accompanied by massive reactive gliosis[19–21]. Although the significance of this alteration is poorly understood, recent findings suggest that modified astroglial functioning might have an important role in the generation and spread of seizure activity. The rest of this section focuses on astrocytic alterations in sclerotic hippocampal seizure foci, which are characteristic of temporal lobe epilepsy (TLE), a condition that has been most extensively investigated in the astroglial field.

K^+ channels and water channels. Neuronal activity leads to transient increases in the extracellular K^+ concentration ($[K^+]_o$), which, if uncorrected, would produce hyperactivity. Clearance of K^+ from the extracellular space is considered to be an important function of astrocytes. Seizure activity *in vivo* is characterized by elevations of $[K^+]_o$ from 3 mM to a ceiling level of 10–12 mM. High $[K^+]_o$, in turn, is sufficient to trigger seizure-like events in acute brain slices. Evidence of the involvement of Kir channels in the impairment of K^+ buffering in sclerotic human hippocampus came from measurements of $[K^+]_o$ using ion-sensitive microelectrodes and patch clamp studies. Heinemann's laboratory compared the effect of barium (Ba^{2+}) on stimulus-induced changes in $[K^+]_o$ in the CA1 region of hippocampal brain slices obtained from patients with Ammon's horn sclerosis (AHS) or lesion-associated epilepsy (non-AHS). In non-AHS tissue, Ba^{2+} significantly augmented the increase in $[K^+]_o$; however, this effect was not observed in AHS tissue. As Ba^{2+} is an inhibitor of Kir channels, these findings suggest that the function of these channels might be impaired in the sclerotic tissue[22]. A comparative patch clamp study[23] (see also REF. 24) provides direct evidence for a down-regulation of Kir currents in the sclerotic human CA1 region of patients with epilepsy. Together, these data suggest that in the sclerotic condition, impaired K^+ buffering through reduced expression of functional Kir channels contributes to, or even initiates, seizure generation: preliminary data indicate that the Kir4.1 subunit might be involved in this process[25].

The coordinated action of Kir4.1 and AQP4 channels in astroglial end-feet, which contact capillaries (BOX 1), suggests that buffering of K^+ through Kir channels is dependent on concomitant transmembrane flux of water in the same cell. In agreement with this hypothesis, impaired K^+ buffering in concert with prolonged seizure duration is observed in *Aqp4*$^{-/-}$ mice[26]. In the sclerotic hippocampus of patients with TLE, AQP4 immunoreactivity of vasculature-associated astrocytic

Box 2 | **Regulation of glutamate transporters in brain disease**

The glial glutamate transporters excitatory amino acid transporter 1 (EAAT1) and EAAT2 carry out the majority of glutamate uptake from the extracellular space in the brain. Antisense, knockdown or pharmacological inhibition of EAAT2 produces epileptiform discharges, neurodegeneration and causes premature death. Reduction of EAAT2 levels was reported in the hippocampus of patients with pharmacoresistant epilepsy. In human epilepsy, downregulation of glutamine synthetase disturbs glutamate conversion and entails accumulation of extracellular and intracellular glutamate. Upregulation of group I metabotropic glutamate receptors (mGluRs) in the brains of patients with epilepsy leads to the release of Ca^{2+} from intracellular stores and downregulation of EAAT1 and EAAT2. In spinal cord astrocytes of patients with sporadic amyotrophic lateral sclerosis (ALS), aberrant splicing of EAAT2 suppresses protein expression. In hereditary ALS with mutations in the mitochondrial Cu/Zn-superoxide dismutase (*SOD1*) gene, reactive oxygen species (ROS) are formed in motor neurons following mitochondrial Ca^{2+} overload. ROS inhibit astroglial EAAT2 activity, which leads to extracellular accumulation of glutamate. ALS is also characterized by elevations in levels of cyclooxygenase 2, which result in prostaglandin E_2 production that is crucial in the control of glutamate release from astrocytes. Enhanced group I mGluR signalling in ALS activates neuronal nitric oxide (NO) synthase, and produces NO and peroxynitrite, leading to the nitration and subsequent inhibition of glial glutamate transporters. During long-term ischaemia, extracellular glutamate levels can increase owing to reversal of glial glutamate transporters. Hyperammonemic conditions after liver failure downregulate EAAT1 expression and elevate glutamate concentrations in the cerebrospinal fluid of patients with hepatic encephalopathy. Cytosolic alkalinization due to ammonia exposure evokes increases in intracellular concentrations of Ca^{2+} and release of glutamate from astrocytes.

end-feet was lower than in non-sclerotic human epileptic hippocampus[27]. This loss of perivascular AQP4 might be a consequence of disruption of the dystrophin complex[28]. In the sclerotic human hippocampus, dislocation of water channels and reduced expression of Kir channels in astrocytes might, therefore, contribute to both impaired K^+ buffering and increased seizure propensity.

Glutamate transporters and receptors. Increased extracellular levels of glutamate have been found in epileptogenic foci[29], and recent work suggests that glutamate transporters and receptors are involved in seizure development and spread. Studies using mice lacking the astroglial transporter GLT1, either owing to genetic deletion[30] or to antisense oligonucleotide-mediated inhibition of protein synthesis[31], revealed that this subtype of transporter is responsible for the majority of extracellular glutamate clearance in the CNS. In mice, knockout of GLT1, but not antisense knockdown, results in spontaneous seizures and hippocampal pathology, which resembles alterations in patients with TLE suffering from AHS. Pharmacological inhibition of GLT1 reduced the threshold for evoking epileptiform activity[32,33] (BOX 2). Reduced expression of GLT1 and GLAST (the other transporter subtype expressed by astrocytes) was also observed in a tuberous sclerosis epilepsy model[34]. However, other animal studies contradict these findings. Tessler and colleagues[35] investigated transporter expression at the mRNA and protein levels in tissues from patients with TLE, but no changes in the expression of EAAT1 or EAAT2 were detected. However, two other groups reported decreased concentrations of EAAT2 as well as reduced[36] or increased[37] EAAT1 immunoreactivity in the sclerotic human hippocampus (BOX 2). The group that showed increased EAAT1 immunoreactivity[37] also noted that EAAT2 was upregulated in the non-sclerotic epileptic hippocampus. These findings support the hypothesis that reduced or dysfunctional glial glutamate transporters in the hippocampus might trigger spontaneous seizures in patients with AHS[38].

The finding that, in patients with TLE, the expression of glutamine synthetase is lower in sclerotic compared with non-sclerotic hippocampus[39] deserves particular consideration. After uptake of glutamate into astrocytes, glutamine synthetase rapidly converts glutamate into glutamine, which is then transported to neurons, where it is converted back to glutamate. Eid and coworkers[39] did not detect any epilepsy-related changes in the expression of EAAT2. They concluded that, in the sclerotic tissue, downregulation of glutamate synthetase slowed the cycle of glutamate–glutamine production and the accumulation of glutamate in astrocytes and the extracellular space[39]. This conclusion is compatible with findings in animal models of epilepsy, and the data that demonstrate slowed glutamate–glutamine cycling in sclerotic human epileptic hippocampus[40]. Whether activation of glutamate transporters, for example, through β-lactam antibiotics[41], might be beneficial in the treatment of epilepsy remains to be seen.

The potential involvement of iGluRs and mGluRs in seizure generation has also been investigated. The GluR2 subunit makes AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors almost impermeable to Ca^{2+}. The molecular determinant for this property of the receptor has been traced to an arginine (R) residue in the pore-forming segment. The codon for arginine is post-transcriptionally introduced into GluR2 mRNA, leading to the substitution of the glutamine (Q) codon by an arginine one in >99% of mRNA molecules — a process known as Q/R site editing. Mouse mutants with deficient GluR2 Q/R editing develop early onset epilepsy with spontaneous and recurrent seizure activity, which suggests that enhanced Ca^{2+} influx through the Q form of the GluR2 subunit of AMPA receptors reduces seizure threshold[42]. Astrocytes also express GluR2, but altered glial GluR2 editing does not seem to have a role in human epilepsy. Each AMPA receptor subunit occurs in two splice forms, termed flip and flop, which have different gating properties. Combined functional and single-cell transcript analyses suggest that enhanced expression of GluR1 flip variants accounts for the prolonged receptor responses observed in hippocampal astrocytes of patients with TLE suffering from AHS[43,44] (FIG. 2). This alteration in the splicing status predicts enhanced depolarization on activation of glial AMPA receptors. Prolonged receptor opening will enhance influx of Ca^{2+} and Na^+. As the latter blocks astroglial Kir channels[45], this would further increase depolarization and reduce the K^+ buffering capacity of astrocytes. It is not clear whether these changes in glial receptor function are causative of, or result from, the epileptic condition. In addition, the question of how and to what extent alterations in glial GluR1 splicing contribute to

Müller glia
A radial-shaped type of astroglial cell that spans the entire thickness of the retina and tightly envelops retinal neurons and neuronal processes. It constitutes the majority of retinal astroglia and is central to retinal development and function.

Ammon's horn sclerosis
(AHS). A histopathological condition often associated with temporal lobe epilepsy, characterized by selective neuronal cell loss in the CA1 and CA4 regions of the hippocampus and reactive sclerosis.

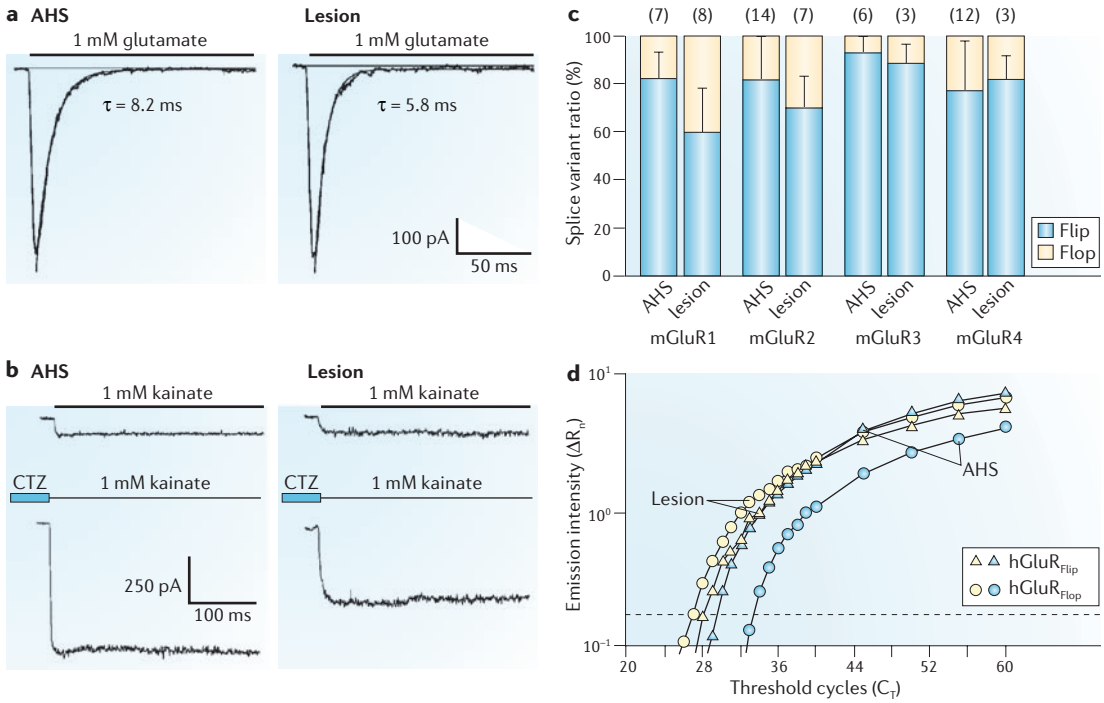


Figure 2 | **Regulation of AMPA receptor splicing in astrocytes of human epileptic hippocampus.** **a** | Fast application of glutamate evoked inward currents in CA1 hippocampal astrocytes from patients with Ammon's horn sclerosis (AHS) and lesion-associated epilepsy (currents were measured at a constant voltage of –70 mV). Note the slowed desensitization (which is indicated by the larger time constant, τ) in AHS cells. **b** | After kainate application, the cells were washed, exposed to cyclothiazide (CTZ), a substance that selectively inhibits desensitization of the flip variants of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors, and then kainate was applied again. In AHS cells, CTZ potentiation was stronger than in the lesioned cells, indicating enhanced expression of flip variants of AMPA receptors in astrocytes from AHS tissue. **c** | To confirm changes in flip/flop splicing of astroglial AMPA receptors in human epilepsy, single cells were isolated from the hippocampus of patients with AHS or lesion-associated epilepsy. After functional characterization, the cytoplasm of the respective cell was collected and analysed with single-cell RT-PCR (reverse transcription-polymerase chain reaction). Restriction analysis revealed prevailing expression of flip versions. The flip variant of the AMPA receptor subunit GluR1 was more abundant in AHS. The number above the columns indicates the number of cells analysed. **d** | Determination of AMPA receptor flip/flop ratios in human astrocytes using semiquantitative real-time PCR. A fluorogenic TaqMan probe was used, yielding an increase in fluorescence emission during progressing transcript amplification. The panel shows amplification plots of one astrocyte — either from patients with AHS (blue) or from those with lesion-associated epilepsy (yellow). Note the higher cycle number necessary to amplify flop transcripts in astrocytes from patients with AHS. The threshold for detecting transcripts of the splice variants was set at a ΔR_n of 0.17 (dashed line). Panels **a** and **b** adapted, with permission, from REF. 48 © (2002) Elsevier Science. Panels **c** and **d** adapted, with permission, from REF. 44 © (2004) Society for Neuroscience.

seizure generation or spread requires further investigation. GluR3 might also have a role in astrocyte-mediated epileptogenesis. This subunit is the target of autoantibodies in Rasmussen's encephalitis — a rare form of childhood epilepsy — and astrocytes are particularly sensitive to these antibodies[46]. Astrocytes cultured from patients with this condition showed spontaneous Ca^{2+} oscillations that were dependent on transmembrane influx of Ca^{2+} (REF. 47). The authors speculated that this might add to neuronal hyperactivity, possibly through autocrine iGluR stimulation by glutamate released from astrocytes.

Under normal conditions, mGluR3 and mGluR5 are the predominant mGluR subtypes expressed by glial cells. Activation of mGluR3 and mGluR5 affects cyclic AMP (cAMP) accumulation and leads to an increase in $[Ca^{2+}]_i$, respectively. In animal models of epilepsy, reactive astrocytes of the hippocampus persistently upregulate mGluR3, mGluR5 and mGluR8 (REF. 48). Electron microscopy studies of hippocampal tissue from patients with TLE show that mGluR2/3, mGluR4 and mGluR8 are expressed in reactive astrocytes[49]. Upregulation of astroglial mGluR2/3 and mGluR5 was also observed in epileptic specimens from patients with focal cortical dysplasia[50]. As their activation modulates the expression of EAAT1 and EAAT2 (REF. 51) and elevates $[Ca^{2+}]_i$, mGluRs in astrocytes might be involved in the formation of seizure foci (BOX 2).

Release of glutamate. Astrocytes are able to release glutamate through a Ca^{2+}-dependent process, which might be involved in seizure generation[52]. Recent work suggests that in chemically induced, acute epilepsy models astrocytes contribute to the generation

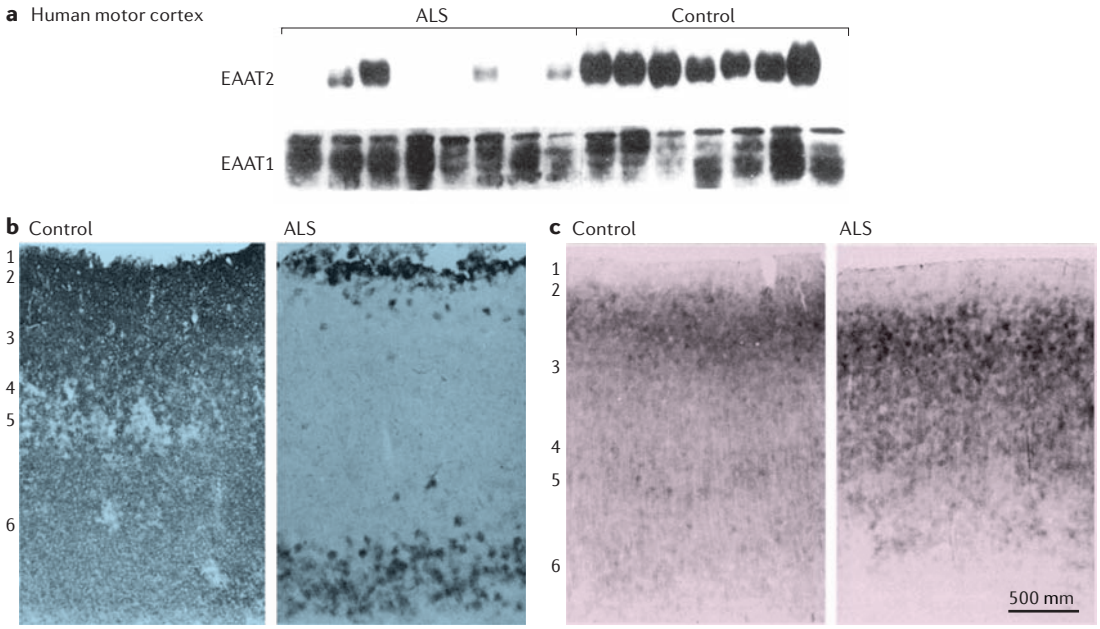


Figure 3 | **Selective loss of excitatory amino acid transporter 2 in human amyotrophic lateral sclerosis.** **a** | Identical aliquots of tissue homogenates from the motor cortex of patients with amyotrophic lateral sclerosis (ALS) or healthy controls were subjected to gel electrophoresis and immunoblotted with antibodies to excitatory amino acid transporter 2 (EAAT2) and EAAT1. Note the selective reduction of EAAT2 protein in ALS sections. **b** | Immunohistochemical localization of the transporter protein EAAT2 in ALS sections and control motor cortex reveals a profound loss of EAAT2 protein in layers 2–5. **c** | By contrast, staining for EAAT1 showed no differences between the control and ALS sections. Adapted, with permission, from REF. 55 © (1995) Wiley InterScience.

of synchronized epileptiform activity[53]. Tian and colleagues[53] provoked epileptic discharges using 4-aminopyridine (a K^+ channel blocker), bicuculline or penicillin ($GABA_A$ receptor antagonists), or bath solutions containing low concentrations of divalent cations. Surprisingly, the neuronal paroxysmal depolarization shifts (PDSs) observed under these conditions were largely insensitive to tetrodotoxin, and also occurred in the absence of synaptic activity. It was also shown that astrocytic increase in $[Ca^{2+}]_i$ is sufficient to stimulate release of glutamate from glial cells, which was crucial for the generation of PDSs. Finally, *in vivo* imaging showed that several antiepileptic drugs suppressed astrocytic Ca^{2+} signalling. It should be noted that human epilepsy is usually associated with significant morphological alterations[19–21] that are absent in the acute animal models. Nevertheless, the findings of Tian *et al.*[53] identify astrocytes as new potential targets for the development of antiepileptic treatments.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset chronic neuromuscular disorder that is characterized by muscle dysfunction caused by selective degeneration of cortical and spinal motor neurons. Worldwide, 95% of all ALS cases are sporadic, and this term could comprise several distinct pathological entities. Astrocytes contribute to the pathophysiology of ALS, particularly through dysfunction of their glutamate transporters, which results in increased extracellular glutamate levels and excitotoxic damage to motor neurons.

Glutamate transporters in amyotrophic lateral sclerosis. Rothstein and colleagues[54] were the first to report reduced glutamate transport in synaptosomes from patients with sporadic ALS. Reduced clearance of extracellular glutamate was disease- and region-specific. Subsequently, immunohistochemistry of tissues from patients with ALS revealed a selective loss of the astroglial glutamate transporter EAAT2 in the motor cortex[55] (FIG. 3) and ventral horn of the spinal cord[56], which closely matched the reduction of motor neurons. As EAAT2 is the main glutamate transporter in the grey matter of the spinal cord[57,58], its loss and subsequent failure of glutamate clearance might be crucial for excitotoxic damage and the pathogenic process of sporadic forms of the disease. Ablation of EAAT2 in mice causes hyperactivity and neuronal cell death[30].

The loss of EAAT2 in ALS was attributed to aberrant RNA splicing, exon skipping and intron retention. The resulting transporter proteins were unstable or had a dominant-negative effect on normally spliced EAAT2. In patients with sporadic ALS, aberrant splicing in astrocytes of the motor cortex and spinal cord might have led to the loss of EAAT2 (REF. 59; BOX 2). However, other studies questioned aberrant EAAT2 splicing as a causative factor in motor neuron degeneration because the phenomenon was also observed in controls and other neurological diseases[60,61].

Oxidative inhibition represents another mechanism that can lead to reductions in transporter expression[62]. Patients with a hereditary form of ALS were found to carry mutations in the gene that encodes Cu/Zn

superoxide dismutase (SOD1)[63], a cytosolic enzyme that converts superoxide radicals to hydrogen peroxide. In a heterologous expression system, SOD1 mutations that are typically found in patients with familial ALS[64] resulted in inhibition of EAAT2 activity (BOX 2). By mutating the *Sod1* gene in mice and rats, animal models for familial ALS were generated that reproduce many aspects of the human phenotype, including loss of EAAT2 (REFS 65,66). In patients with ALS, there is evidence of reduced expression of EAAT2 in astroglia, and damage of astrocytes and motor neurons by oxidative stress, indicating that both mechanisms might interact in the course of the disease[67]. Intriguingly, β-lactam antibiotics stimulate the expression of EAAT2 in SOD1 mutant mice and might provide the basis for developing a new class of neurotherapeutics[41].

In addition to protein damage as a result of oxidative stress, mutant SOD1 might cause toxicity by the aberrant activation of biochemical pathways, accumulation of protein aggregates and stimulation of caspases in motor neurons and astrocytes (for a review, see REF. 68). Interestingly, expression of mutant SOD1 in either motor neurons or astrocytes was not sufficient to induce neuronal degeneration[69–71]. It seems that not only the proportion of mutant-expressing cells but also the topographical relationship between affected neurons and healthy glial cells can influence the progression of the disease[72]. Recent work suggests an alternative ALS pathogenic model. It shows that reactive astrocytes upregulate chromogranin A, which is a component of neurosecretory vesicles that interacts with mutant SOD1 and promotes its secretion. It is possible that increased release of mutant SOD1 might then trigger microgliosis and neuronal death[73].

Neuron–glia interaction. It is unclear whether dysfunction of glial glutamate transporter expression is causative of ALS or whether this dysfunction follows from oxidative damage that is initiated in motor neurons. Rao and colleagues[74] suggest that activation of AMPA receptors might lead to the release of reactive oxygen species from spinal cord motor neurons, which results in oxidation and local decrease of glutamate uptake in neighbouring astrocytes. According to this view, a pathological type of neuron–glia interaction that might contribute to the initiation of ALS is defined by a specific sequence of events — induction of neuronal Ca^{2+} entry through glutamate receptors, followed by mitochondrial Ca^{2+} overload and subsequent production of reactive oxygen species (ROS). ROS then impair glial glutamate uptake, which results in extracellular accumulation of glutamate and eventually exacerbation of Ca^{2+} entry into motor neurons[75,76].

In both ALS mouse models and human ALS, increased expression of cyclooxygenase 2 (COX2) — an enzyme involved in prostaglandin E_2 (PGE_2) synthesis — was observed[77,78]. This finding suggests that another type of neuron–glia signalling interaction might also be involved in pathogenesis of ALS. In neurons, stimulation of NMDA (*N*-methyl-D-aspartate) receptors activates COX2 (REF. 79) and triggers the production of PGE_2 and ROS[80]. PGE_2, in turn, enhances glutamate release from astrocytes[81], which completes the vicious circle of conditions that aggravate the pathological condition (BOX 2). In line with this model is the observation that inhibition of COX2 protects motor neurons from excitotoxic damage[82], presumably by reducing astrocytic glutamate release, which is also COX-inhibitor sensitive.

Activation of mGluRs in astrocytes. Reactive astrocytes in the spinal cord of patients with sporadic or familial ALS have increased immunoreactivity for mGluR1α, mGluR5 and mGluR2/3 (REF. 83). As activation of group I mGluRs triggers an increase in $[Ca^{2+}]_i$, and the subsequent astrocytic glutamate release, it is tempting to speculate that upregulation of these receptors in patients with ALS might enhance neuron–glia communication and exacerbate excitotoxicity-mediated neuronal cell loss. Enhanced mGluR signalling could also lead to the expression of the neuronal NOS splice variants, which have been detected in spinal cord astrocytes of patients with ALS[84]. NO can react with superoxide to form peroxynitrite resulting in the nitration of glutamate transporter proteins, which inhibits their activity[85,86] (BOX 2). By contrast, activation of group II mGluRs stimulates the release of transforming growth factor β (TGFβ), which inhibits neuronal COX2 and could, therefore, be a target for neuroprotective drugs[87].

Stroke and focal cerebral ischaemia

A more than transient deficit in focal cerebral perfusion can result in ischaemic stroke, which is one of the main causes of permanent disability and morbidity, particularly among the older population, and the prevalence of this condition is rapidly growing in Western societies. Typically, insufficient cerebral perfusion results from cardiovascular compromise, emboli or thrombotic occlusions. It has long been known that astroglia restrict, and at the same time mediate, neurovascular contact and communication. Morphologically, perivascular astrocytes are central to neurovascular units, but they are hardly mentioned in the literature relating to neurovascular studies, and their role in neurovascular disease has traditionally been neglected. Three themes seem to have emerged from recent research on glial biology that have potential relevance to neurovascular diseases. First, basic molecular principles that govern the structure and function of the neurovascular link (including astrocytes) have now been elucidated. Second, it has now been ascertained that astrocytes and their reaction to ischaemia are heterogeneous, and further elucidation of this phenomenon at the molecular level should open up new therapeutic approaches. Third, increasing evidence indicates that there are molecular parallels between neural/neuronal and vascular development and maintenance, in which astroglia might have important orchestrating functions.

AQP4, Kir4.1 and CX43 at the vasculo–glial–neuronal junction. In recent years, AQP4 has been recognized as being central — both in terms of function and of molecular integration — to the organization and dynamics of the cellular 'ménage à trois', which defines the neurovascular

Stroke
Tissue damage in the CNS resulting from a lack of adequate blood supply. Stroke is a major cause of death and permanent disability.

Neurovascular unit
Describes a dynamic ensemble comprising neurons, astroglial cells, vascular endothelia, and their associated extracellular matrices. The neurovascular unit is viewed as a modular unit integrating CNS function and perfusion (blood supply).

unit (BOX 1). The high concentrations of this water-permeable channel on perivascular astroglial end-feet provides the molecular evidence that astroglia can be highly polarized, just as neurons can. Precise subcellular localization, rather than absolute levels of AQP4 in astroglia, is crucial for the tissue reaction to focal ischaemia. Removal of the perivascular pool of AQP4 by deletion of α-syntrophin significantly reduced the extent of post-ischaemic oedema[88], similar to that seen after global AQP4 ablation[89]. Intriguingly, α-syntrophin-mediated anchoring of AQP4 is sensitive to ischaemia. In addition, ischaemic challenge of the cerebral cortex results in an almost complete loss of perivascular AQP4 in the ischaemic core, and a substantial reduction in its penumbra, without affecting α-syntrophin distribution[28]. Elucidation of the molecular basis of this phenomenon might provide a target for therapeutic strategies aimed at amelioration of the consequences of cerebral ischaemia. Consistent with the observation that removal of AQP4 from perivascular astrocytic membranes can reduce post-ischaemic oedema is the finding that intracellular redistribution of AQP4, as seen in astrocytes overexpressing endothelin 1, is associated with a propensity to develop more severe oedema, a breakdown of the blood–brain-barrier and tissue damage following ischaemia[90]. This study also identified endothelin 1, which might be derived from reactive astrocytes (see below), as a potential regulator of AQP4. However, a caveat to be considered is that the transgenic approach used in the study, although providing an *in vivo* model, results in increased levels of cerebral endothelin 1, which is not seen in patients with equivalent pathophysiological conditions.

As outlined in BOX 1, normal volume regulation in glia seems to require cooperation of AQP4 and Kir4.1 activities. Strikingly, as observed in the retina, ablation of AQP4 alone attenuates the development of oedema, but coordinated dysregulation of AQP4 and Kir4.1 enhances the sensitivity to ischaemic insults[91,92]. This raises the issue regarding to what extent protracted ischaemic tissue swelling and damage might be perceived as a consequence of dysregulated expression and cellular localization of AQP4 and its partner, Kir4.1, and whether this might be targeted therapeutically. In this context, the observation that astrocytes are heterogeneous with respect to Kir channel expression deserves further consideration.

Functional and molecular evidence suggests that several types of Kir channel in different astroglial populations coexist. As an example, astrocytes in the forebrain and olfactory bulb express heteromeric channels assembled from Kir4.1 and Kir5.1, whereas hippocampal and thalamic astrocytes lack Kir5.1 (REF. 93). In addition, members of the Kir2 and Kir6 families have been found in astrocytes of various brain regions[45,94,95]. It is not clear to what extent different Kir subunits contribute to K^+ buffering of astrocytes, and so far systematic quantitative studies that correlate these data with expression of AQP4 are not available.

Another mechanism that has attracted considerable interest as a determinant of post-ischaemic tissue damage is gap junctional coupling. Although the discussion of a neuroprotective role of astrocytic gap junctions is still controversial[96], the weight of the evidence, which includes observations in a recently described mouse stroke model in which expression of CX43 was selectively silenced in astrocytes, suggests that connexin expression in glia might help to contain stroke-related tissue damage[97,98]. A recent study implicates a potential coupling between the expression of CX43 and that of AQP4, such that downregulation of AQP4 entails a concomitant reduction in CX43 expression[99]. However, this work was conducted in cultured astrocytes and did not study molecular interactions that are presumed to occur typically in ischaemic foci. Therefore, there is also *in vitro* evidence that, in contrast to its positive effects on AQP4 expression, endothelin 1 can inhibit CX43 expression and gap junction coupling in glia[100]. Although these observations pin-point molecular constituents of astrocytes that might be perturbed in ischaemia, the functional significance *in vivo* is unclear. In this context, it is interesting to note that connexin-mediated protective effects on injured tissues might not require functional gap junctions[101]. In particular, there is an ongoing and unresolved debate (for example, see REF. 102) regarding whether functions related to connexin expression are due to concomitant changes in cell–cell coupling or reflect hemichannel-mediated release of neurotransmitters. So far, the latter has been documented under non-physiological conditions (for example, see REF. 103), and the occurrence of this mechanism *in vivo* remains to be shown.

Astroglia and extracellular glutamate levels. It is generally recognized that the breakdown of cell membrane integrity and cellular metabolism, which result from ischaemia-induced energy shortage, as well as the fragmentation of extracellular space (BOX 1), can lead to an unwarranted exposure of neurons to glutamate, and glutamate-associated excitotoxicity would at least exacerbate the damage caused by energy starvation. The almost exclusive expression of EAAT1 and EAAT2 in glia and the crucial role of astroglia in physiological glutamate cycling (see above) seem to put astrocytes at the centre of excitotoxic ischaemic insult. Different regions of the brain express different levels of EAAT1 and EAAT2 (REF. 37), which has been associated with their distinct sensitivities to ischaemia[104]. However, our understanding of the roles of glial glutamate transporters in the pathophysiological progression of ischaemic insult remains fragmented[105].

The importance of EAAT2 for post-ischaemic extracellular glutamate levels has been analysed using gene knockout[106,107], antisense ablation[108] and pharmacological blockade[109] techniques. These studies have identified a clear effect of EAAT2 on extracellular glutamate dynamics, but it remains to be determined what this might mean for the post-ischaemic tissue reaction. Antisense knockdown of EAAT2 expression was found to negatively influence post-ischaemic infarct volume, neurological outcome and mortality rate in rats[108]. By contrast, pharmacological blockade of EAAT2 did not appreciably affect hippocampal tissue damage[109]. One

possible explanation for this discrepancy might be that EAAT2 expression is low in the hippocampus (in particular in the hippocampal CA1 field[104]), and therefore this transporter might be less relevant, or its dysfunction harder to detect, than in other parts of the brain.

A point that might be particularly important regarding the development of post-ischaemic therapeutic strategies is the observation that extracellular glutamate levels might be differentially affected by EAAT2 activity at various time points after the initiating insult[107]. In the first 5 min of ischaemia, concentrations of extracellular glutamate were higher in EAAT2-knockout mice compared with controls; however, the reverse was true when glutamate levels were measured 10–20 min after ischaemia[107]. These data have been interpreted to indicate that, in early ischaemia, EAAT2 takes up extracellular glutamate (which would have a protective effect), whereas it releases astroglial glutamate — possibly as a result of the conversion of glutamate to glutamine — during protracted ischaemia, thereby contributing to neuronal toxicity (BOX 2).

As the post-ischaemic period is prolonged, additional regulatory mechanisms might be involved to further modulate glial glutamate handling. Therefore, both the uncoupling from neuronal input[110,111] and systemic stress reaction[112,113] have been reported as having a role in the regulation of EAAT2 expression. Intriguingly, astrocytes in distinct regions of the brain seem to respond differently to these stimuli[111,113].

Beyond the finding that the net effect of glial glutamate handling on neuronal pathology might vary temporally after ischaemia, it has recently been shown that glial glutamate handling also differs spatially around lesion areas — that is, in the ischaemic tissue proper versus its penumbra. So, although reversal of EAAT2 activity was described as a key mechanism for glutamate release in severely ischaemic regions, glutamate release in incompletely ischaemic regions seems to be mediated, at least for the most part, by volume-activated anion channels (also known as volume-sensitive osmolyte and anion channels (VSOACs))[114]. The molecular identity of these channels has yet to be resolved, but ClC3 (a ClC-type chloride channel) has been proposed as a candidate[115]. Indeed, recent evidence implicates functional heterogeneity of VSOACs (for an expanded discussion, see REF. 116). The protective effect of tamoxifen in models of cerebral ischaemia has been related to its suppressive effect on VSOAC-mediated release of excitatory amino acid transmitters[117,118], although this agent also inhibits neuronal NOS and scavenges free radicals[119] (for a review, see REF. 120).

The question concerning whether patients with stroke could profit from, or rather be further injured by, interference with glial glutamate transport, might, therefore, be too simplistic. At a minimum, any approaches of this nature must consider the progressive nature of ischaemic damage, both in time and in space. Furthermore, the clinical significance of the observations mentioned above might be complicated by the recent finding[121] that expression of glutamate transporters in glia might differ significantly among species and even within one given region. Differential ('patchy') expression of glutamate transporters in astrocytes seems to be characteristic of primates, including humans. As such, the pathophysiological significance of these diverse mechanisms is currently hard to fathom or generalize, as their involvement might be different depending on which regions of the brain are injured, and could vary with respect to the precipitating injury.

Astroglia and post-ischaemic tissue repair. Several recent studies have pointed out conceptual, mechanistic and molecular parallels that underlie vasculogenesis and neuronal wiring (for reviews, see REFS 122,123). Intriguingly, some of the key players that have been identified as impinging on both vasculogenesis and neuronal wiring are expressed in astroglial cells, and are subject to pathophysiological and/or physiological regulation. This indicates that astroglia might be important in maintaining the integrity and plasticity of the neurovascular unit, as well as in defining its reactions in disease. Astrocytes, and reactive astrocytes in particular, have, therefore, been identified as a source and potential target of endothelins[124,125], vascular endothelial growth factor (VEGF)[126,127] and angiopoietin 1 (REF. 126) — three factors that are prominently involved in glia-mediated vasculogenesis[127], neurogenesis[128], directed migration of neural precursors[129], neuritogenesis[130,131] and glial plasticity.

The role of these factors in the progression and/or repair of ischaemic insult is only now about to become apparent and some factors might have multiple, contrasting actions. For example, endothelin-mediated vasospasm might reduce bleeding, but at the same time it should exacerbate hypoperfusion. Blockade of astrocytic endothelin A receptors protects astrocytes from ischaemic injury[132]. Conversely, overexpression of endothelin 1 has deleterious effects on the ischaemic brain through dysregulation of astrocytic AQP4 expression[90].

The vascular effects of endothelins, VEGF and angiopoietin in the context of stroke and focal cerebral ischaemia have resulted in the formulation of therapeutic concepts based on interference with their signalling[133]. The emerging perception that they also directly affect glia and neurons should prove essential to the improvement of such approaches.

Hepatic encephalopathy

Hepatic encephalopathy is a neurological disorder that might accompany both acute and chronic liver failure. Patients with hepatic encephalopathy show neuropsychiatric symptoms including alterations of mood and personality, cognitive impairment, convulsions and coma. The high mortality rate of the disease results from the formation of brain oedema and cerebral herniation. Prominent neuropathological features include astrocyte swelling (in acute liver failure) and Alzheimer type II astrocytosis (in chronic liver failure), which is characterized by swollen cell bodies with large pale nuclei, altered glial fibrillary acidic protein expression and impaired cell metabolism.

Volume-sensitive osmolyte and anion channel
(VSOAC; also known as VRAC). A molecularly so far undefined channel that, on cell swelling, results in an outward rectifying, electrogenic flow of Cl^- and mediates a passive efflux of osmolytes.

Brain oedema in hepatic encephalopathy. Hepatic insufficiency results in hyperammonaemia, which can result in a substantial increase (up to millimolar) in ammonium ion concentration in the cerebrospinal fluid (CSF). Ammonium ion concentrations of the same order of magnitude are also observed in congenital conditions in which there are deficits in enzymes involved in the urea cycle. Ammonia and ammonium ions seem to enter astrocytes by diffusion, via carriers and through Kir channels[134]. There, it serves as a substrate of the astroglial enzyme glutamine synthetase in a chemical reaction that consumes ATP. In hyperammonaemic rats, inhibition of glutamine synthetase reduces brain oedema[135] and attenuates astrocyte hypertrophy and swelling[136]. However, the role of glutamine synthetase, particularly in protracted hepatic encephalopathy, might be more complex. In hepatic encephalopathy animal models, the activity of glutamine synthetase is differentially regulated in regions that contain mainly excitatory versus inhibitory synapses. This, together with the fact that its subcellular distribution is altered in diseased tissues[137], indicates that this enzyme might be neuroprotective. Although it is not clear how these observations might be mechanistically reconciled, they provide compelling experimental evidence for a key role of astrocytes in the pathogenesis of hepatic encephalopathy[138].

Astroglia-mediated cerebral glutamine accumulation perturbs control of K^+ homeostasis, and hyperammonaemia was found to increase cortical $[K^+]_o$ by several millimolar[139]. Moreover, *in vitro* culture studies show that ammonia can also increase astrocytic expression of AQP4 (REF. 140). However, although hyperammonaemia interferes with K^+ homeostasis and astrocytic expression of AQP4 — two systems that are central to cerebral volume regulation (BOX 1) — available data are insufficient for the formulation of a consistent model of how hyperammonaemia mechanistically causes brain oedema.

Astrocytic glutamate uptake and synaptic transmission. Glutamate uptake is reduced in cultured astrocytes after ammonia challenge[141]. This inhibitory action of ammonia might also operate *in vivo*, as downregulation of EAAT1 mRNA and protein expression has been observed in the frontal cortex of hyperammonaemic rats and in rats with acute liver failure[142,143] (BOX 2). The reduced glutamate uptake capacity of astrocytes during hyperammonaemia correlates with elevated glutamate concentrations in the CSF, which have been described in patients with hepatic encephalopathy, as well as in hepatic encephalopathy animal models; the increase in glutamate release might, in turn, induce NMDA receptor-mediated excitotoxicity[144–146].

Another potential source of elevated extracellular glutamate levels is astrocytic transmitter release. As mentioned above, neurotransmitter release might be mediated by Ca^{2+}-independent and Ca^{2+}-dependent mechanisms. Exposure of cultured astrocytes to ammonium ions induces changes in cytosolic pH, and increases in $[Ca^{2+}]_i$ and in Ca^{2+}-dependent glutamate releases (BOX 2). It has been estimated that this type of transmitter release would be sufficient to cause hyperexcitation *in vivo*[147]. However, interpretation of data obtained in more intact preparations (for example, brain slices) is more complicated. In these preparations, acute application of ammonia can affect glutamatergic and GABA-mediated neurotransmission by different mechanisms[148]. Ammonium ions can have an inhibitory effect on excitatory synaptic transmission. This presumably comes about through inhibition of the neuronal enzyme glutaminase, which leads to a decrease in glutamate concentrations in presynaptic terminals. In addition, ammonium ions can also induce prolonged depolarization and thereby block action potentials, as well as reduce efficacy of postsynaptic glutamate receptors. Inhibitory postsynaptic currents were also reduced by ammonium ions, possibly as a result of lowered excitatory input onto interneurons or elevated $[K^+]_o$, which impeded Cl^- extrusion and led to a shift in the Cl^- equilibrium potential. In addition, synaptic transmission in acute liver failure is compromised by downregulation of cortical AMPA and kainate receptors[149]. In conclusion, although the role of astrocytes in hepatic encephalopathy has been well studied, a useful molecular target to interfere with their obvious dysfunction has not yet been identified.

Conclusion and future perspectives

During the past few years, detailed molecular and functional characterization of astroglial cells indicate that these cells are active players in the CNS, rather than mere inert 'brain glue'[15]. This new perspective has rekindled the question regarding the role of astroglial cells in neurological diseases. Might astrocytes orchestrate disease-related mechanisms as do radial glia[150] in normal neural development? Indeed, astroglia, and in particular reactive astroglia (which are activated by nerve injury), express various regulatory molecules, such as endothelins, VEGF and ephrins/Eph-receptors, which are known to modulate neuronal development, axonal growth and vasculogenesis[133,151]. This raises the issue of whether regulation and/or correction of a 'misled' astroglial reaction could be used as 'master-switch' to simultaneously control a host of pathogenic reactions.

Although a vast body of evidence has documented astroglial dysfunction, and even dysregulation of astroglial-specific functions in various neurological diseases, it is still premature to construe a unifying picture from these data. This might be, at least in part, due to the fact that the term 'astrocyte' covers a heterogeneous group of cells, including highly polarized and differentiated cells as well as stem cells, which makes comparison of individual studies difficult. Indeed, molecular, functional and structural definition of astroglial heterogeneity is a rapidly evolving field, and a better definition of astroglial subtypes should greatly promote our understanding of their specific roles in pathophysiology. Progress in the field during the past few years indicates that this could lead to the development of novel cell-centred therapeutic approaches to neurological disorders.

1. Parpura, V. *et al*. Glutamate-mediated astrocyte–neuron signalling. *Nature* **369**, 744–747 (1994).
2. Steinhäuser, C., Jabs, R. & Kettenmann, H. Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. *Hippocampus* **4**, 19–35 (1994).
3. Jabs, R. *et al*. Synaptic transmission onto hippocampal glial cells with hGFAP promoter activity. *J. Cell Sci.* **118**, 3791–3803 (2005).
4. Matthias, K. *et al*. Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. *J. Neurosci.* **23**, 1750–1758 (2003).
5. Wallraff, A., Odermatt, B., Willecke, K. & Steinhäuser, C. Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. *Glia* **48**, 36–43 (2004).
6. Verkhratsky, A. & Steinhäuser, C. Ion channels in glial cells. *Brain Res. Rev.* **32**, 380–412 (2000).
7. Kettenmann, H. & Steinhäuser, C. In *Neuroglia* 2nd edn (eds Kettenmann, H. & Ransom, B. R.) 131–145 (Oxford Univ. Press, 2005).
8. Higashi, K. *et al*. An inwardly rectifying K^+ channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. *Am. J. Physiol. Cell Physiol.* **281**, C922–C931 (2001).
9. Verkman, A. S. More than just water channels: unexpected cellular roles of aquaporins. *J. Cell Sci.* **118**, 3225–3232 (2005).
10. Amiry-Moghaddam, M., Frydenlund, D. S. & Ottersen, O. P. Anchoring of aquaporin-4 in brain: molecular mechanisms and implications for the physiology and pathophysiology of water transport. *Neuroscience* **129**, 999–1010 (2004).
11. Theis, M., Sohl, G., Eiberger, J. & Willecke, K. Emerging complexities in identity and function of glial connexins. *Trends Neurosci.* **28**, 188–195 (2005).
12. Nedergaard, M., Ransom, B. & Goldman, S. A. New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci.* **26**, 523–530 (2003).
13. Danbolt, N. C. Glutamate uptake. *Prog. Neurobiol.* **65**, 1–105 (2001).
14. Bergles, D. E., Diamond, J. S. & Jahr, C. E. Clearance of glutamate inside the synapse and beyond. *Curr. Opin. Neurobiol.* **9**, 293–298 (1999).
15. Volterra, A. & Meldolesi, J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nature Rev. Neurosci.* **6**, 626–640 (2005).
16. Zonta, M. *et al*. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nature Neurosci.* **6**, 43–50 (2003).
17. Mulligan, S. J. & MacVicar, B. A. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* **431**, 195–199 (2004).
18. Takano, T. *et al*. Astrocyte-mediated control of cerebral blood flow. *Nature Neurosci.* **9**, 260–267 (2006).
19. Kim, J. H. Pathology of epilepsy. *Exp. Mol. Pathol.* **70**, 345–367 (2001).
20. Blümcke, I., Thom, M. & Wiestler, O. D. Ammon's horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. *Brain Pathol.* **12**, 199–211 (2002).
21. De Lanerolle, N. C. & Lee, T. S. New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. *Epilepsy Behav.* **7**, 190–203 (2005).
22. Kivi, A. *et al*. Effects of barium on stimulus-induced rises of $[K^+]_o$ in human epileptic non-sclerotic and sclerotic hippocampal area CA1. *Eur. J. Neurosci.* **12**, 2039–2048 (2000).
23. Hinterkeuser, S. *et al*. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. *Eur. J. Neurosci.* **12**, 2087–2096 (2000).
References 22 and 23 show the downregulation of inwardly rectifying K^+ currents and the impairment of Ba^{2+}-sensitive K^+-buffering in the sclerotic hippocampus from epilepsy patients.
24. Bordey, A. & Sontheimer, H. Properties of human glial cells associated with epileptic seizure foci. *Epilepsy Res.* **32**, 286–303 (1998).
25. Schröder, W. *et al*. Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. *Epilepsia* **41**, S181–S184 (2000).
26. Binder, D. K. *et al*. Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. *Glia* (in the press).
27. Eid, T. *et al*. Loss of perivascular aquaporin 4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. *Proc. Natl Acad. Sci. USA* **102**, 1193–1198 (2005).
Reports that in patients with AHS the density of AQP4 water channels at perivascular end-feet is reduced. The perturbed water flux through astrocytes entails impaired buffering of extracellular potassium.
28. Amiry-Moghaddam, M. *et al*. An α-syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc. Natl Acad. Sci. USA* **100**, 2106–2111 (2003).
Using a mouse model with defective anchoring of AQP4 to the cytoskeleton, the authors document the importance of proper subcellular segregation for aquaporin function in astrocytes under ischaemic stress *in vivo*.
29. Glass, M. & Dragunow, M. Neurochemical and morphological changes associated with human epilepsy. *Brain Res. Rev.* **21**, 29–41 (1995).
30. Tanaka, K. *et al*. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* **276**, 1699–1702 (1997).
31. Rothstein, J. D. *et al*. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* **16**, 675–686 (1996).
32. Campbell, S. L. & Hablitz, J. J. Glutamate transporters regulate excitability in local networks in rat neocortex. *Neuroscience* **127**, 625–635 (2004).
33. Demarque, M. *et al*. Glutamate transporters prevent the generation of seizures in the developing rat neocortex. *J. Neurosci.* **24**, 3289–3294 (2004).
34. Wong, M. *et al*. Impaired glial glutamate transport in a mouse tuberous sclerosis epilepsy model. *Ann. Neurol.* **54**, 251–256 (2003).
35. Tessler, S., Danbolt, N. C., Faull, R. L. M., Storm-Mathisen, J. & Emson, P. C. Expression of the glutamate transporters in human temporal lobe epilepsy. *Neuroscience* **88**, 1083–1091 (1999).
36. Mathern, G. W. *et al*. Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. *Neurology* **52**, 453–472 (1999).
37. Proper, E. A. *et al*. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain* **125**, 32–43 (2002).
38. During, M. J. & Spencer, D. D. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* **341**, 1607–1610 (1993).
39. Eid, T. *et al*. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet* **363**, 28–37 (2004).
40. Petroff, O. A., Errante, L. D., Rothman, D. L., Kim, J. H. & Spencer, D. D. Glutamate–glutamine cycling in the epileptic human hippocampus. *Epilepsia* **43**, 703–710 (2002).
References 39 and 40 indicate that in epilepsy in humans downregulation of astroglial glutamine synthetase disturbs the conversion of glutamate and its accumulation in the extracellular space.
41. Rothstein, J. D. *et al*. β-Lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* **433**, 73–77 (2005).
Identifies β-lactam antibiotics as potential therapeutic agents for the treatment of diseases associated with excitotoxic degeneration through upregulation of the astroglial glutamate transporter EAAT2.
42. Brusa, R. *et al*. Early-onset epilepsy and postnatal lethality associated with an editing-deficient *GluR-B* allele in mice. *Science* **270**, 1677–1680 (1995).
43. Seifert, G. *et al*. Changes in flip/flop splicing of astroglial AMPA receptors in human temporal lobe epilepsy. *Epilepsia* **43** (Suppl. 5), 162–167 (2002).
44. Seifert, G., Hüttmann, K., Schramm, J. & Steinhäuser, C. Enhanced relative expression of glutamate receptor 1 flip AMPA receptor subunits in hippocampal astrocytes of epilepsy patients with Ammon's horn sclerosis. *J. Neurosci.* **24**, 1996–2003 (2004).
The authors show that aberrant AMPA receptor splicing in astrocytes of the sclerotic human hippocampus affects the kinetics of AMPA receptor desensitization, which might contribute to seizure generation.
45. Schröder, W., Seifert, G., Hüttmann, K., Hinterkeuser, S. & Steinhäuser, C. AMPA receptor-mediated modulation of inward rectifier K^+ channels in astrocytes of mouse hippocampus. *Mol. Cell Neurosci.* **19**, 447–458 (2002).
46. Whitney, K. D. & McNamara, J. O. GluR3 autoantibodies destroy neural cells in a complement-dependent manner modulated by complement regulatory proteins. *J. Neurosci.* **20**, 7307–7316 (2000).
47. Manning, T. J. Jr & Sontheimer, H. Spontaneous intracellular calcium oscillations in cortical astrocytes from a patient with intractable childhood epilepsy (Rasmussen's encephalitis). *Glia* **21**, 332–337 (1997).
48. Steinhäuser, C. & Seifert, G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. *Eur. J. Pharmacol.* **447**, 227–237 (2002).
49. Tang, F. R. & Lee, W. L. Expression of the group II and III metabotropic glutamate receptors in the hippocampus of patients with mesial temporal lobe epilepsy. *J. Neurocytol.* **30**, 137–143 (2001).
50. Aronica, E. *et al*. Expression and cell distribution of group I and group II metabotropic glutamate receptor subtypes in taylor-type focal cortical dysplasia. *Epilepsia* **44**, 785–795 (2003).
51. Aronica, E. *et al*. Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: opposite regulation of glutamate transporter proteins. *Eur. J. Neurosci.* **17**, 2106–2118 (2003).
52. Kang, N., Xu, J., Xu, Q., Nedergaard, M. & Kang, J. Astrocytic glutamate release-induced transient depolarization and epileptiform discharges in hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* **94**, 4121–4130 (2005).
53. Tian, G. F. *et al*. An astrocytic basis of epilepsy. *Nature Med.* **11**, 973–981 (2005).
Reports that, in acute epilepsy models, glutamate released from astrocytes causes paroxysmal depolarization shifts in neurons. Several antiepileptic drugs were shown to reduce astrocytic $[Ca^{2+}]_i$ elevations and therefore astrocytes might be considered as new targets for antiepileptic treatments.
54. Rothstein, J. D., Martin, L. J. & Kuncl, R. W. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N. Engl. J. Med.* **326**, 1464–1468 (1992).
55. Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J. & Kuncl, R. W. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann. Neurol.* **38**, 73–84 (1995).
References 54 and 55 describe the loss of glial glutamate transporters in ALS and identify astrocytes as crucial mediators of excitotoxicity and motor neuron loss.
56. Sasaki, S., Komori, T. & Iwata, M. Excitatory amino acid transporter 1 and 2 immunoreactivity in the spinal cord in amyotrophic lateral sclerosis. *Acta Neuropathol. (Berl.)* **100**, 138–144 (2000).
57. Yamada, K. *et al*. Glutamate transporter GLT-1 is transiently localized on growing axons of the mouse spinal cord before establishing astrocytic expression. *J. Neurosci.* **18**, 5706–5713 (1998).
58. Schmitt, A., Asan, E., Puschel, B., Jons, T. & Kugler, P. Expression of the glutamate transporter GLT1 in neural cells of the rat central nervous system: non-radioactive *in situ* hybridization and comparative immunocytochemistry. *Neuroscience* **71**, 989–1004 (1996).
59. Lin, C. L. *et al*. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron* **20**, 589–602 (1998).
Shows that in patients with ALS, loss of astroglial EAAT2 is due to aberrant RNA splicing.
60. Meyer, T. *et al*. The RNA of the glutamate transporter EAAT2 is variably spliced in amyotrophic lateral sclerosis and normal individuals. *J. Neurol. Sci.* **170**, 45–50 (1999).
61. Honig, L. S., Chambliss, D. D., Bigio, E. H., Carroll, S. L. & Elliott, J. L. Glutamate transporter EAAT2 splice variants occur not only in ALS, but also in AD and controls. *Neurology* **55**, 1082–1088 (2000).
62. Volterra, A., Trotti, D., Tromba, C., Floridi, S. & Racagni, G. Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *J. Neurosci.* **14**, 2924–2932 (1994).

63. Rosen, D. R. *et al*. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62 (1993).
First report that mutation of the Cu/Zn superoxide dismutase (*SOD1*) gene mediates neurodegeneration in hereditary ALS.
64. Trotti, D., Rolfs, A., Danbolt, N. C., Brown, R. H. Jr & Hediger, M. A. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nature Neurosci.* **2**, 427–433 (1999).
Shows that mutant SOD1 leads to neurotoxicity through inactivation of the astroglial glutamate transporter EAAT2.
65. Howland, D. S. *et al*. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc. Natl Acad. Sci. USA* **99**, 1604–1609 (2002).
66. Bruijn, L. I. *et al*. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* **18**, 327–338 (1997).
67. Pedersen, W. A. *et al*. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. *Ann. Neurol.* **44**, 819–824 (1998).
68. Bruijn, L. I., Miller, T. M. & Cleveland, D. W. Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu. Rev. Neurosci.* **27**, 723–749 (2004).
69. Pramatarova, A., Laganiere, J., Roussel, J., Brisebois, K. & Rouleau, G. A. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J. Neurosci.* **21**, 3369–3374 (2001).
70. Lino, M. M., Schneider, C. & Caroni, P. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J. Neurosci.* **22**, 4825–4832 (2002).
71. Gong, Y. H., Parsadanian, A. S., Andreeva, A., Snider, W. D. & Elliott, J. L. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J. Neurosci.* **20**, 660–665 (2000).
72. Clement, A. M. *et al*. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* **302**, 113–117 (2003).
73. Urushitani, M. *et al*. Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. *Nature Neurosci.* **9**, 108–118 (2006).
Suggests that, in ALS, astrocytes contribute to the secretion of mutant SOD1, which, in turn, produces microgliosis and motor neuron death.
74. Rao, S. D., Yin, H. Z. & Weiss, J. H. Disruption of glial glutamate transport by reactive oxygen species produced in motor neurons. *J. Neurosci.* **23**, 2627–2633 (2003).
75. Rao, S. D. & Weiss, J. H. Excitotoxic and oxidative cross-talk between motor neurons and glia in ALS pathogenesis. *Trends Neurosci.* **27**, 17–23 (2004).
76. Levine, J. B., Kong, J. M., Nadler, M. & Xu, Z. S. Astrocytes interact intimately with degenerating motor neurons in mouse amyotrophic lateral sclerosis (ALS). *Glia* **28**, 215–224 (1999).
77. Almer, G. *et al*. Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. *Ann. Neurol.* **49**, 176–185 (2001).
78. Yasojima, K., Tourtellotte, W. W., McGeer, E. G. & McGeer, P. L. Marked increase in cyclooxygenase-2 in ALS spinal cord: implications for therapy. *Neurology* **57**, 952–956 (2001).
79. Yamagata, K., Andreasson, K. I., Kaufmann, W. E., Barnes, C. A. & Worley, P. F. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron* **11**, 371–386 (1993).
80. Pepicelli, O. *et al*. *In vivo* activation of *N*-methyl-D-aspartate receptors in the rat hippocampus increases prostaglandin E_2 extracellular levels and triggers lipid peroxidation through cyclooxygenase-mediated mechanisms. *J. Neurochem.* **81**, 1028–1034 (2002).
81. Bezzi, P. *et al*. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* **391**, 281–285 (1998).
82. Drachman, D. B. *et al*. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann. Neurol.* **52**, 771–778 (2002).
83. Aronica, E., Catania, M. V., Geurts, J., Yankaya, B. & Troost, D. Immunohistochemical localization of group I and II metabotropic glutamate receptors in control and amyotrophic lateral sclerosis human spinal cord: upregulation in reactive astrocytes. *Neuroscience* **105**, 509–520 (2001).
84. Catania, M. V., Aronica, E., Yankaya, B. & Troost, D. Increased expression of neuronal nitric oxide synthase spliced variants in reactive astrocytes of amyotrophic lateral sclerosis human spinal cord. *J. Neurosci.* **21**, RC148 (2001).
Suggests that in human ALS aberrant expression of neuronal NOS in reactive astrocytes is involved in pathogenesis.
85. Trotti, D. *et al*. Peroxynitrite inhibits glutamate transporter subtypes. *J. Biol. Chem.* **271**, 5976–5979 (1996).
86. Beal, M. F. *et al*. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. *Ann. Neurol.* **42**, 644–654 (1997).
87. Bruno, V. *et al*. Metabotropic glutamate receptor subtypes as targets for neuroprotective drugs. *J. Cereb. Blood Flow Metab.* **21**, 1013–1033 (2001).
88. Amiry-Moghaddam, M. *et al*. α-Syntrophin deletion removes the perivascular but not endothelial pool of aquaporin-4 at the blood–brain barrier and delays the development of brain edema in an experimental model of acute hyponatremia. *FASEB J.* **18**, 542–544 (2004).
89. Manley, G. T. *et al*. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nature Med.* **6**, 159–163 (2000).
90. Lo, A. C. *et al*. Endothelin-1 overexpression leads to further water accumulation and brain edema after middle cerebral artery occlusion via aquaporin 4 expression in astrocytic end-feet. *J. Cereb. Blood Flow Metab.* **25**, 998–1011 (2005).
91. Da, T. & Verkman, A. S. Aquaporin-4 gene disruption in mice protects against impaired retinal function and cell death after ischemia. *Invest. Ophthalmol. Vis. Sci.* **45**, 4477–4483 (2004).
92. Dalloz, C. *et al*. Targeted inactivation of dystrophin gene product Dp71: phenotypic impact in mouse retina. *Hum. Mol. Genet.* **12**, 1543–1554 (2003).
93. Hibino, H., Fujita, A., Iwai, K., Yamada, M. & Kurachi, Y. Differential assembly of inwardly rectifying K^+ channel subunits, Kir4.1 and Kir5.1, in brain astrocytes. *J. Biol. Chem.* **279**, 44065–44073 (2004).
94. Thomzig, A. *et al*. Kir6.1 is the principal pore-forming subunit of astrocyte but not neuronal plasma membrane K–ATP channels. *Mol. Cell. Neurosci.* **18**, 671–690 (2001).
95. Stonehouse, A. H. *et al*. Characterisation of Kir2.0 proteins in the rat cerebellum and hippocampus by polyclonal antibodies. *Histochem. Cell Biol.* **112**, 457–465 (1999).
96. Farahani, R. *et al*. Alterations in metabolism and gap junction expression may determine the role of astrocytes as 'good samaritans' or executioners. *Glia* **50**, 351–361 (2005).
97. Nakase, T., Söhl, G., Theis, M., Willecke, K. & Naus, C. C. Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin43 in astrocytes. *Am. J. Pathol.* **164**, 2067–2075 (2004).
98. Perez Velazquez, J. L., Kokarovtseva, L., Sarbaziha, R., Jeyapalan, Z. & Leshchenko, Y. Role of gap junctional coupling in astrocytic networks in the determination of global ischaemia-induced oxidative stress and hippocampal damage. *Eur. J. Neurosci.* **23**, 1–10 (2006).
99. Nicchia, G. P. *et al*. New possible roles for aquaporin-4 in astrocytes: cell cytoskeleton and functional relationship with connexin43. *FASEB J.* **19**, 1674–1676 (2005).
100. Rozyczka, J., Figiel, M. & Engele, J. Chronic endothelin exposure inhibits connexin43 expression in cultured cortical astroglia. *J. Neurosci. Res.* **79**, 303–309 (2005).
101. Lin, J. H. *et al*. Connexin mediates gap junction-independent resistance to cellular injury. *J. Neurosci.* **23**, 430–441 (2003).
102. Takano, T. *et al*. Receptor-mediated glutamate release from volume sensitive channels in astrocytes. *Proc. Natl Acad. Sci. USA* **102**, 16466–16471 (2005).
103. Ye, Z. C., Wyeth, M. S., Baltan-Tekkok, S. & Ransom, B. R. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J. Neurosci.* **23**, 3588–3596 (2003).
104. Fujita, H., Sato, K., Wen, T. C., Peng, Y. & Sakanaka, M. Differential expressions of glycine transporter 1 and three glutamate transporter mRNA in the hippocampus of gerbils with transient forebrain ischemia. *J. Cereb. Blood Flow Metab.* **19**, 604–615 (1999).
105. Maragakis, N. J. & Rothstein, J. D. Glutamate transporters in neurologic disease. *Arch. Neurol.* **58**, 365–370 (2001).
106. Hamann, M., Rossi, D. J., Marie, H. & Attwell, D. Knocking out the glial glutamate transporter GLT-1 reduces glutamate uptake but does not affect hippocampal glutamate dynamics in early simulated ischaemia. *Eur. J. Neurosci.* **15**, 308–314 (2002).
107. Mitani, A. & Tanaka, K. Functional changes of glial glutamate transporter GLT-1 during ischemia: an *in vivo* study in the hippocampal CA1 of normal mice and mutant mice lacking GLT-1. *J. Neurosci.* **23**, 7176–7182 (2003).
108. Rao, V. L. *et al*. Antisense knockdown of the glial glutamate transporter GLT-1, but not the neuronal glutamate transporter EAAC1, exacerbates transient focal cerebral ischemia-induced neuronal damage in rat brain. *J. Neurosci.* **21**, 1876–1883 (2001).
109. Selkirk, J. V. *et al*. Role of the GLT-1 subtype of glutamate transporter in glutamate homeostasis: the GLT-1-preferring inhibitor WAY-855 produces marginal neurotoxicity in the rat hippocampus. *Eur. J. Neurosci.* **21**, 3217–3228 (2005).
110. Swanson, R. A. *et al*. Neuronal regulation of glutamate transporter subtype expression in astrocytes. *J. Neurosci.* **17**, 932–940 (1997).
111. Schluter, K., Figiel, M., Rozyczka, J. & Engele, J. CNS region-specific regulation of glial glutamate transporter expression. *Eur. J. Neurosci.* **16**, 836–842 (2002).
112. Reagan, L. P. *et al*. Chronic restraint stress up-regulates GLT-1 mRNA and protein expression in the rat hippocampus: reversal by tianeptine. *Proc. Natl Acad. Sci. USA* **101**, 2179–2184 (2004).
113. Zschocke, J. *et al*. Differential promotion of glutamate transporter expression and function by glucocorticoids in astrocytes from various brain regions. *J. Biol. Chem.* **280**, 34924–34932 (2005).
114. Feustel, P. J., Jin, Y. & Kimelberg, H. K. Volume-regulated anion channels are the predominant contributors to release of excitatory amino acids in the ischemic cortical penumbra. *Stroke* **35**, 1164–1168 (2004).
115. Yamamoto-Mizuma, S. *et al*. Altered properties of volume-sensitive osmolyte and anion channels (VSOACs) and membrane protein expression in cardiac and smooth muscle myocytes from *Clcn3*$^{-/-}$ mice. *J. Physiol.* **557**, 439–456 (2004).
116. Jentsch, T. J., Stein, V., Weinreich, F. & Zdebik, A. A. Molecular structure and physiological function of chloride channels. *Physiol. Rev.* **82**, 503–568 (2002).
117. Kimelberg, H. K. *et al*. Acute treatment with tamoxifen reduces ischemic damage following middle cerebral artery occlusion. *Neuroreport* **11**, 2675–2679 (2000).
118. Zhang, Y. *et al*. Behavioral and histological neuroprotection by tamoxifen after reversible focal cerebral ischemia. *Exp. Neurol.* **196**, 41–46 (2005).
119. Osuka, K., Feustel, P. J., Mongin, A. A., Tranmer, B. I. & Kimelberg, H. K. Tamoxifen inhibits nitrotyrosine formation after reversible middle cerebral artery occlusion in the rat. *J. Neurochem.* **76**, 1842–1850 (2001).
Suggests that tamoxifen is an inhibitor of Ca^{2+}/calmodulin-dependent NOS and the subsequent peroxynitrite production in transient focal cerebral ischaemia, which might represent one mechanism for the neuroprotective effect of tamoxifen.
120. Kimelberg, H. K. Astrocytic swelling in cerebral ischemia as a possible cause of injury and target for therapy. *Glia* **50**, 389–397 (2005).
121. Williams, S. M. *et al*. Glial glutamate transporter expression patterns in brains from multiple mammalian species. *Glia* **49**, 520–541 (2005).
122. Serini, G. & Bussolino, F. Common cues in vascular and axon guidance. *Physiology (Bethesda)* **19**, 348–354 (2004).
123. Carmeliet, P. & Tessier-Lavigne, M. Common mechanisms of nerve and blood vessel wiring. *Nature* **436**, 193–200 (2005).
124. Tsang, M. C., Lo, A. C., Cheung, P. T., Chung, S. S. & Chung, S. K. Perinatal hypoxia-/ischemia-induced endothelin-1 mRNA in astrocyte-like and endothelial cells. *Neuroreport* **12**, 2265–2270 (2001).

125. Ehrenreich, H. The astrocytic endothelin system: toward solving a mystery focus on 'distinct pharmacological properties of ET-1 and ET-3 on astroglial gap junctions and Ca^{2+} signaling'. *Am. J. Physiol.* **277**, C614–C615 (1999).
126. Acker, T., Beck, H. & Plate, K. H. Cell type specific expression of vascular endothelial growth factor and angiopoietin-1 and -2 suggests an important role of astrocytes in cerebellar vascularization. *Mech. Dev.* **108**, 45–57 (2001).
127. West, H., Richardson, W. D. & Fruttiger, M. Stabilization of the retinal vascular network by reciprocal feedback between blood vessels and astrocytes. *Development* **132**, 1855–1862 (2005).
128. Sun, Y. *et al*. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J. Clin. Invest.* **111**, 1843–1851 (2003).
129. Zhang, H., Vutskits, L., Pepper, M. S. & Kiss, J. Z. VEGF is a chemoattractant for FGF-2-stimulated neural progenitors. *J. Cell Biol.* **163**, 1375–1384 (2003).
130. Kosacka, J. *et al*. Angiopoietin-1 promotes neurite outgrowth from dorsal root ganglion cells positive for Tie-2 receptor. *Cell Tissue Res.* **320**, 11–19 (2005).
131. Ward, N. L., Putoczki, T., Mearow, K., Ivanco, T. L. & Dumont, D. J. Vascular-specific growth factor angiopoietin 1 is involved in the organization of neuronal processes. *J. Comp. Neurol.* **482**, 244–256 (2005).
132. Danielyan, L. *et al*. The blockade of endothelin A receptor protects astrocytes against hypoxic injury: common effects of BQ-123 and erythropoietin on the rejuvenation of the astrocyte population. *Eur. J. Cell Biol.* **84**, 567–579 (2005).
133. Fagan, S. C., Hess, D. C., Hohnadel, E. J., Pollock, D. M. & Ergul, A. Targets for vascular protection after acute ischemic stroke. *Stroke* **35**, 2220–2225 (2004).
134. Ott, P. & Larsen, F. S. Blood–brain barrier permeability to ammonia in liver failure: a critical reappraisal. *Neurochem. Int.* **44**, 185–198 (2004).
135. Takahashi, H., Koehler, R. C., Brusilow, S. W. & Traystman, R. J. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. *Am. J. Physiol.* **261**, H825–H829 (1991).
136. Willard-Mack, C. L. *et al*. Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. *Neuroscience* **71**, 589–599 (1996).
137. Suarez, I., Bodega, G. & Fernandez, B. Glutamine synthetase in brain: effect of ammonia. *Neurochem. Int.* **41**, 123–142 (2002).
138. Butterworth, R. F. Pathophysiology of hepatic encephalopathy: a new look at ammonia. *Metab. Brain Dis.* **17**, 221–227 (2002).
139. Sugimoto, H., Koehler, R. C., Wilson, D. A., Brusilow, S. W. & Traystman, R. J. Methionine sulfoximine, a glutamine synthetase inhibitor, attenuates increased extracellular potassium activity during acute hyperammonemia. *J. Cereb. Blood Flow Metab.* **17**, 44–49 (1997).
140. Rama Rao, K. V., Chen, M., Simard, J. M. & Norenberg, M. D. Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. *Neuroreport* **14**, 2379–2382 (2003).
141. Bender, A. S. & Norenberg, M. D. Effects of ammonia on L-glutamate uptake in cultured astrocytes. *Neurochem. Res.* **21**, 567–573 (1996).
142. Norenberg, M. D., Huo, Z., Neary, J. T. & Roig-Cantesano, A. The glial glutamate transporter in hyperammonemia and hepatic encephalopathy: relation to energy metabolism and glutamatergic neurotransmission. *Glia* **21**, 124–133 (1997).
143. Knecht, K., Michalak, A., Rose, C., Rothstein, J. D. & Butterworth, R. F. Decreased glutamate transporter (GLT-1) expression in frontal cortex of rats with acute liver failure. *Neurosci. Lett.* **229**, 201–203 (1997).
144. Watanabe, A. *et al*. Glutamic acid and glutamine levels in serum and cerebrospinal fluid in hepatic encephalopathy. *Biochem. Med.* **32**, 225–231 (1984).
145. Michalak, A., Rose, C., Butterworth, J. & Butterworth, R. F. Neuroactive amino acids and glutamate (NMDA) receptors in frontal cortex of rats with experimental acute liver failure. *Hepatology* **24**, 908–913 (1996).
146. Monfort, P., Munoz, M. D., ElAyadi, A., Kosenko, E. & Felipo, V. Effects of hyperammonemia and liver failure on glutamatergic neurotransmission. *Metab. Brain Dis.* **17**, 237–250 (2002).
147. Rose, C., Kresse, W. & Kettenmann, H. Acute insult of ammonia leads to calcium-dependent glutamate release from cultured astrocytes, an effect of pH. *J. Biol. Chem.* **280**, 20937–20944 (2005).
Shows that hyperammonaemia produces an increase in $[Ca^{2+}]_i$ and triggers glial glutamate release in cultured astrocytes. This mechanism might contribute to disorders associated with glutamate-dependent overexcitation.
148. Szerb, J. C. & Butterworth, R. F. Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. *Prog. Neurobiol.* **39**, 135–153 (1992).
149. Michalak, A. & Butterworth, R. F. Selective loss of binding sites for the glutamate receptor ligands [^{3}H]kainate and (S)-[^{3}H]5-fluorowillardiine in the brains of rats with acute liver failure. *Hepatology* **25**, 631–635 (1997).
150. Götz, M. Glial cells generate neurons — master control within CNS regions: developmental perspectives on neural stem cells. *Neuroscientist* **9**, 379–397 (2003).
151. Eichmann, A., Makinen, T. & Alitalo, K. Neural guidance molecules regulate vascular remodeling and vessel navigation. *Genes Dev.* **19**, 1013–1021 (2005).
152. Binder, D. K., Papadopoulos, M. C., Haggie, P. M. & Verkman, A. S. *In vivo* measurement of brain extracellular space diffusion by cortical surface photobleaching. *J. Neurosci.* **24**, 8049–8056 (2004).
153. Uckermann, O. *et al*. Glutamate-evoked alterations of glial and neuronal cell morphology in the guinea pig retina. *J. Neurosci.* **24**, 10149–10158 (2004).
154. Izumi, Y. *et al*. Swelling of Müller cells induced by AP3 and glutamate transport substrates in rat retina. *Glia* **17**, 285–293 (1996).
155. MacVicar, B. A., Feighan, D., Brown, A. & Ransom, B. Intrinsic optical signals in the rat optic nerve: role for K^+ uptake via NKCC1 and swelling of astrocytes. *Glia* **37**, 114–123 (2002).
156. Strange, K. Cellular volume homeostasis. *Adv. Physiol. Educ.* **28**, 155–159 (2004).
157. Parkerson, K. A. & Sontheimer, H. Biophysical and pharmacological characterization of hypotonically activated chloride currents in cortical astrocytes. *Glia* **46**, 419–436 (2004).
158. Nielsen, S. *et al*. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J. Neurosci.* **17**, 171–180 (1997).
159. Nagelhus, E. A. *et al*. Immunogold evidence suggests that coupling of K^+ siphoning and water transport in rat retinal Müller cells is mediated by a coenrichment of Kir4.1 and AQP4 in specific membrane domains. *Glia* **26**, 47–54 (1999).
160. Niermann, H., Amiry-Moghaddam, M., Holthoff, K., Witte, O. W. & Ottersen, O. P. A novel role of vasopressin in the brain: modulation of activity-dependent water flux in the neocortex. *J. Neurosci.* **21**, 3045–3051 (2001).
161. Amiry-Moghaddam, M. *et al*. Delayed K^+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of α-syntrophin-null mice. *Proc. Natl Acad. Sci. USA* **100**, 13615–13620 (2003).
162. Nagelhus, E. A., Mathiisen, T. M. & Ottersen, O. P. Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. *Neuroscience* **129**, 905–913 (2004).
163. Papadopoulos, M. C. & Verkman, A. S. Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis. *J. Biol. Chem.* **280**, 13906–13912 (2005).
164. Bloch, O., Papadopoulos, M. C., Manley, G. T. & Verkman, A. S. Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. *J. Neurochem.* **95**, 254–262 (2005).
165. Simard, M., Arcuino, G., Takano, T., Liu, Q. S. & Nedergaard, M. Signaling at the gliovascular interface. *J. Neurosci.* **23**, 9254–9262 (2003).

Acknowledgements
The authors wish to thank D. Binder for comments on the manuscript. Work of the authors is supported by Deutsche Forschungsgemeinschaft. We apologize to all those whose work could not be discussed due to space constraints.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
AQP4 | COX2 | Cx30 | Cx43 | EAAT1 | EAAT2 | Endothelin 1 | $GABA_A$ receptor | $GABA_B$ receptor | GLAST | GLT1 | GluR1 | GluR2 | GluR3 | Kir4.1 | Kir5.1 | mGluR3 | mGluR4 | mGluR5 | mGluR8 | *SOD1*
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ALS | Temporal lobe epilepsy

FURTHER INFORMATION
Department of Experimental Neurobiology: http://www.meb.uni-bonn.de/neurochirurgie/exp_neurobiologie
Access to this interactive links box is free online.